Obesity Is Associated with *BRAF*^{V600E}-Mutated Thyroid Cancer

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Background: Thyroid cancer incidence has increased in many parts of the world since the 1980s, as has the prevalence of obesity. Evidence suggests that people with greater body size have higher thyroid cancer risk. However, it is unclear whether this association is causal or is driven by over-diagnosis of indolent cancers, because overweight/obese people use health services more frequently than those of normal weight, thus conferring greater opportunity for incidental diagnosis. Assessing whether obesity is associated with higher-risk thyroid cancers might help clarify this issue.

Methods: We recruited 1013 people diagnosed with thyroid cancer between 2013 and 2016 and 1057 population controls, frequency matched by sex and age group. We used logistic regression to assess the association between body mass index (BMI) and overall thyroid cancer risk as well as by tumor *BRAF* mutational status as a marker of potentially higher-risk cancer.

Results: Overall, obesity was associated with greater risk of thyroid cancer (odds ratio [OR]=1.72; 95% confidence interval [CI 1.37–2.16] for obese vs. normal BMI). The association with obesity was significantly stronger for *BRAF*-mutation positive than *BRAF*-negative papillary thyroid cancers (PTCs; OR=1.71 [CI 1.17–2.50] for *BRAF*-positive vs. *BRAF*-negative cancers). The increased risks associated with overweight/obesity did not vary by histological subtypes or presence/absence of adverse tumor histologic features.

Conclusions: Greater risk of *BRAF*-mutated PTCs among those with high BMI suggests that the association may not merely reflect greater health care service use and indicates an independent relationship between obesity and clinically important thyroid cancer.

Keywords: body mass index, obesity, over-diagnosis, *BRAF* mutation, thyroid cancer

Introduction

THYROID CANCER INCIDENCE has risen sharply in many high-income countries over the past three decades. Although there is evidence that greater ascertainment of indolent tumors (“over-diagnosis”) has contributed to this rise (1,2), there may also have been a true increase in disease occurrence (3). One possible driver of a true increase in differentiated thyroid cancer (DTC) incidence could be increasing body fatness, as the prevalence of overweight and obesity has also risen over a similar period. Several studies have examined the relationship between obesity (measured by body mass index [BMI]) and thyroid cancer risk. Most (4–

9), but not all (10,11), suggest that obesity is associated with increased thyroid cancer risk in both women and men. However, it is possible that the observed association does not reflect a biological relationship between obesity and thyroid cancer but instead could reflect more frequent use of health care services by people who are overweight or obese (12,13), with consequent increased opportunity for ascertainment of indolent thyroid lesions.

Examining whether obesity is associated with thyroid cancers that have higher-risk features could help disentangle the effects of over-diagnosis from a true association. A positive association between BMI and cancers of greater clinical relevance could indicate a true causal relationship, whereas if

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BMI is more strongly related to thyroid cancers that are probably indolent, this might imply that over-diagnosis is driving the association. The B-type Raf kinase- (*BRAF*)^{V600E} mutation, considered an early mutational event in the development of some papillary thyroid cancers (PTCs) (14), has consistently been associated with adverse prognostic features such as extrathyroidal extension and lymph node metastases (15,16). Thus, *BRAF* mutation status, alongside traditional histopathological features, may be a useful marker to differentiate between indolent tumors and those that are potentially of greater clinical significance (17). A few studies have assessed whether etiology varies by mutational subtype in thyroid cancer, although two hospital-based case-only analyses from Korea and China suggested that *BRAF* mutations were more common in tumors from patients with higher BMI compared with those in the normal weight range (18,19).

In this study, we used data from a large population-based case-control study to investigate the association between BMI and risk of DTC overall and to assess whether any observed associations varied by histological subtype, *BRAF* mutational status, or the presence of potentially adverse histological features.

Materials and Methods

We used information from the Queensland Thyroid Cancer Study (QTCS) to assess the association between BMI and DTC risk. The QTCS is a case-control study of thyroid cancer that recruited participants between July 2013 and August 2016. At recruitment, all participants were residents of the Australian state of Queensland and aged 18 to 79 years.

The process of case ascertainment is described elsewhere (20). Briefly, people diagnosed with DTC were ascertained through two sources: The Royal Brisbane and Women's Hospital (RBWH) and the Queensland Cancer Registry (QCR). Postsurgical thyroid cancer care is very centralized in Queensland. The majority of people diagnosed with thyroid cancer in Queensland are reviewed by RBWH clinicians either in person or via telephone consultation because it is the only institution in the state providing radioactive iodine, a key postoperative treatment for thyroid cancer. Those who were potentially eligible for the study were approached at their clinic appointment and invited to participate. We additionally used the population-based QCR to identify all other newly diagnosed thyroid cancer patients who were either not approached at their clinic appointment or not reviewed there; these people were invited to participate using the Registry's standard procedures. A questionnaire to self-complete, along with a study information sheet, consent form, and reply-paid envelope were mailed to those who agreed to participate. Cancer registration is a legal requirement in Australia, so ascertainment of all cases of thyroid cancer diagnosed in Queensland during the study period was essentially complete.

Overall, 703 potential case participants were approached at the RBWH; of them, 8% ($n=60$) declined participation and 5% ($n=34$) were ineligible based on either their diagnosis date, age, inability to complete the questionnaire in English, or their tumor type. Of the remaining 609, 595 (98%) sent back the completed study questionnaire. We additionally

identified 865 newly diagnosed thyroid cancer patients through the QCR; treating doctors gave permission to approach 766 of them (89%). Of these, 17 were not eligible, 53 refused participation, 245 did not respond, and 33 did not complete the study questionnaire, leaving 418 participants. Combining the cases from RBWH and QCR, 1013 people with thyroid cancer participated in the study (American Association for Public Opinion Research [AAPOR] response rate—71%).

Control participants, frequency matched to the cases by age group and sex, were recruited by using the Queensland Electoral Roll as the sampling frame. Voting is compulsory in Australia, so the Electoral roll provides a close-to-complete population-based sampling frame. Potential control participants were initially approached by letter and then, where possible, were telephoned to determine their eligibility and interest in participating in the study. Those who were eligible and willing to participate were sent the same materials as the case participants.

Invitation for participation were sent to 5651 randomly selected potential controls, and 2301 of them responded to the invitation. Of the contactable potential controls ($n=2301$), 42% ($n=972$) refused further contact and 9% ($n=203$) declined to participate after initial consent. Another 65 people were ineligible because they had their thyroid removed, were too sick to participate, were aged older than 80 years at contact, or were unable to complete the questionnaire in English. We excluded four people, as they did not return their consent form. Overall, 1057 potential control participants met the eligibility criteria and sent back the completed questionnaire (AAPOR response rate—47%). Both the case and control participants gave written consent to take part in the study, and approval was obtained from the Human Research Ethics Committee (HREC) of the QIMR Berghofer Medical Research Institute.

All participants completed a health and lifestyle questionnaire, which asked about a range of exposures including medical conditions, medication use, reproductive factors, smoking, alcohol, physical activity, and body size. Participants were asked to report their body weight at the reference date (diagnosis date for cases and consent date for controls), at one year before the reference date, and when they were aged around 20 years (for those who were older than 20 years of age). We also asked them to record their height. We calculated BMI by dividing weight (in kilograms) by height (in meters) squared and then categorized the participants into four groups according to World Health Organization criteria: underweight (BMI <18.5), normal weight ($18.5 \leq \text{BMI} < 25.0$), overweight ($25.0 \leq \text{BMI} < 30.0$), and obese (BMI ≥ 30.0) (21). There were a few underweight people in the study (1.6% of cases and 2% of controls), so they were grouped with those of normal weight for the analysis. To reduce the possibility of reverse causation (cancer affecting BMI), we used the reported BMI one year before the reference date to assess the association.

Participants were categorized as never smokers, former smokers, or current smokers. We calculated pack-years of smoking by using the reported number of cigarettes smoked per day and the total number of years of smoking. Consumption of alcohol was categorized into three groups: life-long nondrinker, former drinker, and current drinker. In addition, we determined the quantity of alcohol consumed

by using the number of standard drinks per week. We also asked questions about physical activity (number of sessions of walking, moderate and strenuous physical activity). Participants' residential postcodes were used to determine their index of relative socioeconomic disadvantage (IRSD) score, an area-based measure that summarizes a range of socioeconomic conditions of the people and households residing in an area. A low IRSD score implies that the person lives in an area of relatively greater disadvantage, while a high score indicates lower rates of disadvantage in an area (22).

We obtained participants' permission to access their Medicare Benefit Schedule (MBS) records and used those to calculate the number of consultations with medical practitioners in the preceding 4.5 years. Medicare is the scheme through which all Australian permanent residents receive subsidized health care services outside the public hospital system (medical consultations, pathology testing, and medical imaging). We also obtained case participants' consent to access their clinical records and tissue sections. We extracted information about tumor characteristics such as histological subtype, extra-thyroidal tumor extension, and lymph node metastases from pathology records. Tissue sections were stained by using the BenchMark ULTRA platform (Ventana Medical Systems, Inc., Tucson, AZ) to determine the *BRAF*^{V600E} mutational status of each thyroid tumor. Specialist endocrine pathologists scored the slides as either *BRAF* positive, negative, or indeterminate.

Statistical analysis

We compared the distributions of personal characteristics of the controls to case participants, both overall and according to their *BRAF* status. Logistic regression models with thyroid cancer as the outcome were used to estimate the odds ratios (ORs), as an approximation of the relative risk, and 95% confidence interval (CI) for the association between thyroid cancer and BMI. We also assessed the association in female and male separately to see whether the association differed by sex. Only a small number of participants ($n = 21$, ~1% of 2070) had missing exposure or covariate information; thus, we used complete-case analysis to estimate the associations (23,24). BMI associations were modeled in categories and as a continuous measure. To investigate possible nonlinearity of the association with the continuous measure of BMI, we used generalized additive models with fixed degrees of freedom (degrees of freedom = 3). Statistical significance of the nonlinear parameter was assessed by comparing models' deviances with and without a nonlinear term for BMI.

We constructed directed acyclic graphs based on the literature and expert opinion to determine the potential confounders (factors that are antecedents of both exposure and outcome, but do not lie on the causal pathway) to include in the multivariable models. The association between BMI and thyroid cancer risk was adjusted for age (continuous), sex, educational attainment (school only, technical training, university degree), smoking status (never, former, current), alcohol-drinking status (never, former, current), number of walking sessions per week (0, 1–2, 3–4, 5 or more), history of benign thyroid disease (yes, no), and IRSD score (continuous). Additional adjustment for moderate and strenuous physical activity, pack years of smoking, and number of

standard drinks per week did not change the estimates by more than 10%, so they were not included in the final model.

To assess whether more frequent contact with medical practitioners might mediate the association between obesity and thyroid cancer risk, we performed a counterfactual mediation analysis (25) considering binary forms of the exposure (underweight/normal vs. overweight/obese) and the potential mediator variable (≤ 8 visits/year vs. > 8 visits/year). Because of the nonlinear association between frequency of consultation with medical practitioners and thyroid cancer risk, we used the binary form of the mediator (divided at the median) in the analysis instead of its continuous form. We estimated the controlled direct effect (CDE), natural direct effect (NDE), natural indirect effect (NIE), total effect (TE), and proportions mediated, thus allowing for an exposure-mediator interaction (26,27). The CDE expresses the average causal effect on thyroid cancer risk if everyone changed BMI category (everyone is underweight/normal vs. everyone is overweight/obese), and where the frequency of visit to physicians is controlled at a fixed value uniformly in the population. The NDE also expresses the average causal effect on thyroid cancer risk for the change in BMI, but with the frequency of visits to physicians fixed at a level it would have taken if everyone was underweight/normal. The NIE captures the average causal effect on thyroid cancer risk if the BMI level is fixed (everyone is overweight/obese), and the frequency of visits to physicians for each individual changes from the level it would take if everyone is underweight/normal to the level it would take if everyone is overweight/obese. Finally, the TE expresses the overall average change in thyroid cancer risk for the change in BMI and the proportion mediated measures the extent to which TEs of obesity on thyroid cancer risk operate through frequent consultations with medical practitioners (26,27). We also assessed whether the percentage mediated changes when we excluded services that were accessed in the six months before reference date.

We used multinomial logistic regression (controls and two case groups) to investigate whether the relationship between BMI and DTC varied by tumor characteristics, including: (i) histological subtype (PTC or follicular thyroid cancer [FTC]); (ii) *BRAF* mutation status (positive or negative, among PTCs only since *BRAF* mutation rarely, if ever, occurs in FTCs); or (iii) presence or absence of potentially adverse histological features (extrathyroidal tumor extension, lymph node metastasis, or lymphovascular invasion).

Quantitative bias analyses

To assess the possibility that our results were affected by selection bias resulting from a sub-optimal participation proportion among selected controls, we re-estimated the association between BMI and thyroid cancer by applying the age- and sex-specific BMI distribution of the Queensland general population (reported in the Australian Health Survey 2011–12) (28) to our control participants and then used adjusted proportions to calculate bias-corrected ORs and CIs. We repeated the analysis cited earlier by stratifying the cases according to their *BRAF* mutational status. We then considered the potential for selection bias among our case participants. As we had no equivalent population with which to make comparisons, we calculated estimates by assuming that

the proportion who were overweight and obese was 5% higher in nonparticipating cases compared with participating cases and incorporated these in our quantitative bias analysis (QBA).

Results

The majority of the 2070 (1057 controls, 1013 cases) study participants were women (73%), and the median age was 52 years (inter-quartile range 42–63; men slightly older than women: 56 vs. 51 years). Because we used a frequency-matched design, the age and sex distributions were similar across cases and controls. Most tumors (~88%) were of papillary subtype. Tumor tissue sections were retrieved for 902 participants; 799 had PTC, and *BRAF* mutation immunohistochemistry staining produced definitive results (either positive or negative) in 757 (95%) of the papillary lesions. Of the tumor sections with definitive *BRAF* results, 59% ($n=444$) were mutation positive. The age and sex distributions were similar across *BRAF*-positive and -negative cases. The characteristics of control and case participants overall and by *BRAF* mutational status are shown in Table 1.

Table 2 shows the crude and adjusted odds ratios (OR_{adj}) for the association between BMI one year before the reference date and thyroid cancer risk overall (21 participants with missing exposure/covariate information were excluded, leaving 2049 in the analysis). Being overweight or obese was associated with increased risk of thyroid cancer ($OR_{adj}=1.52$ [CI 1.22–1.90] and $OR_{adj}=1.72$ [CI 1.37–2.16], respectively) compared with being in the normal weight range. The magnitude of these associations was slightly smaller in females than in males, although the interaction between sex and BMI was not statistically significant for either the categorical or the continuous BMI measure. With BMI as a continuous measure, each 5 kg/m² increment was associated with 11% ($OR_{adj}=1.11$ [CI 1.03–1.20]) and 18% ($OR_{adj}=1.18$ [CI 0.99–1.41]) higher risk of thyroid cancer among females and males, respectively. The test for nonlinearity in the overall effect of BMI was statistically significant (test for nonlinearity $p=0.001$, Supplementary Fig. S1); however, the nonlinear pattern appeared to be driven by a very small number of participants with extremely high BMI (BMI >50; $n=18$). Excluding these observations, the nonlinear BMI effect was no longer statistically significant (test for nonlinearity, $p=0.21$).

Our counterfactual mediation analysis suggested that 15% of the observed overweight/obesity-thyroid cancer association might be mediated by more frequent contact with medical practitioners by those who were obese or overweight (OR_{adj} of NIE=1.06 [CI 1.02–1.11]), although the direct effect (NDE) of the overweight/obesity-thyroid cancer relationship was still statistically significant ($OR_{adj}=1.52$ [CI 1.25–1.86]). The proportion of the effect mediated by frequent consultations was larger in females than in males (19% vs. 8%) (Supplementary Table S1). However, when the consultations with medical practitioners recorded in the six months before the reference date were excluded (those more likely to be associated with diagnostic work-up), very little of the overall association was mediated (Supplementary Table S2).

Figure 1 shows the association between overweight/obesity and thyroid cancer by tumor characteristics. Obesity

was associated with slightly higher odds of PTC than FTC ($OR_{adj}=1.71$ vs. 1.57, Fig. 1 and Supplementary Table S3), although the estimates for FTC were not statistically significant. When we stratified the group with PTC by the *BRAF* mutational status of their tumors, we found that the association with obesity was elevated in both *BRAF* subtypes ($OR_{adj}=2.20$ and 1.28 for *BRAF*-positive and *BRAF*-negative cancers, respectively), with results similar in females and males (Fig. 1 and Table 3). Changing the reference category of the multinomial regression to *BRAF*-negative cancer indicated that the association with obesity was statistically significantly stronger for *BRAF*-positive than *BRAF*-negative PTC ($OR_{adj}=1.71$ [CI 1.17–2.50], for *BRAF*-positive vs. *BRAF*-negative cancers; p -value=0.005). Further, exclusion of tumors <10 mm in diameter did not alter these results substantially (Supplementary Table S4). In addition, our mediation analysis suggested that the proportion of the overweight/obesity-thyroid cancer association mediated through frequent contact with medical practitioners was lower for those with *BRAF*-positive tumors than *BRAF*-negative tumors (12% and 22%, respectively; Supplementary Table S5), although the proportion mediated was attenuated in both subtypes when services recorded in the six months before the reference date were excluded (Supplementary Table S6). Despite the differences by *BRAF* status, we found that the association with obesity was similar in those having tumors with and without potentially adverse histological features ($OR_{adj}=1.73$ and 1.70, respectively, Fig. 1; Supplementary Table S7) even after we restricted the case sample to those with PTC. The similarity in risks may have been due to moderate to high prevalence of *BRAF*-mutated tumors in both groups (49% and 77% in tumors without and with adverse tumor features, respectively; Supplementary Table S8).

Quantitative bias analysis

The results of our QBA are presented in Supplementary Tables S9 and S10. The proportion of control participants who were obese was lower than reported in a survey of the general Queensland population (28) (25.1% vs. 30.5% for females; 26.8% vs. 36.4% for male), suggesting the potential for our results to be affected by selection bias. When we adjusted the BMI distribution of our control participants to reflect that of the Queensland population, the overall obesity-thyroid cancer association was attenuated and no longer statistically significant in either females (bias-corrected $OR=1.19$ [CI 0.93–1.52]) or males (bias-corrected $OR=0.93$ [CI 0.58–1.50]) (Supplementary Table S9). However, when we restricted the analysis to *BRAF*-mutated PTC, the association remained elevated and statistically significant in females but not in males (bias-corrected OR s in women and men were 1.55 [CI 1.13–2.12] and 1.06 [CI 0.56–2.00], respectively; Supplementary Table S10). In addition, 30% of our eligible cases did not participate, which could also have induced some degree of selection bias. When we conservatively assumed that the proportion who were overweight and obese was 5% higher in nonparticipating compared with participating cases, then the overall estimate for the association with obesity was 1.38 [CI 1.10–1.73] for females and 1.13 [CI 0.72–1.78] for males (Supplementary Table S9).

TABLE 1. CHARACTERISTICS OF THE CONTROL AND CASE PARTICIPANTS

Characteristics	Controls (n = 1057) n (%)	Cases (n = 1013) n (%)	PTC cases ^a	
			BRAF-positive cases (n = 444) n (%)	BRAF-negative cases (n = 313) n (%)
Age (years) at reference date ^b				
<30	63 (6.0)	66 (6.5)	26 (5.9)	26 (8.3)
30–39	157 (14.9)	154 (15.2)	64 (14.4)	55 (17.6)
40–49	212 (20.1)	215 (21.2)	96 (21.6)	68 (21.7)
50–59	274 (25.9)	275 (27.2)	131 (29.5)	82 (26.2)
60–69	226 (21.4)	194 (19.2)	84 (18.9)	55 (17.6)
70–79	125 (11.8)	109 (10.8)	43 (9.7)	27 (8.6)
Median (IQR)	53 (42–63)	52 (41–62)	52 (42–61)	51 (39–60)
Sex				
Female	785 (74.3)	730 (72.1)	330 (74.3)	236 (75.4)
Male	272 (25.7)	283 (27.9)	114 (25.7)	77 (24.6)
BMI one year before reference date				
Underweight/normal (BMI <25.0)	441 (41.9)	314 (31.2)	123 (27.9)	114 (36.7)
Overweight (25.0 ≤ BMI <30.0)	344 (32.7)	353 (35.0)	152 (34.5)	110 (35.4)
Obese (BMI ≥30.0)	267 (25.4)	341 (33.8)	166 (37.6)	87 (28.0)
Missing	5	5	3	2
Median (IQR)	26.1 (22.9–30.1)	27.4 (23.8–31.9)	28.1 (24.5–32.9)	26.7 (23.2–30.6)
Educational attainment				
School only	393 (37.2)	396 (39.1)	172 (38.7)	121 (38.7)
Technical training	260 (24.6)	298 (29.4)	132 (29.7)	77 (24.6)
University training	404 (38.2)	319 (31.5)	140 (31.5)	115 (36.7)
Smoking status				
Never smoker	610 (57.7)	578 (57.1)	264 (59.5)	177 (56.6)
Current smoker	112 (10.6)	107 (10.6)	42 (9.5)	44 (14.1)
Former smoker	335 (31.7)	328 (32.4)	138 (31.1)	92 (29.4)
Alcohol drinking status				
Nondrinker	125 (11.8)	138 (13.6)	65 (14.6)	37 (11.8)
Current drinker	848 (80.2)	762 (75.2)	331 (74.6)	249 (79.6)
Former drinker	84 (8.0)	113 (11.2)	48 (10.8)	27 (8.6)
Walking sessions per week				
0	206 (19.6)	258 (25.5)	127 (28.7)	66 (21.2)
1–2	186 (17.7)	178 (17.6)	84 (19.0)	57 (18.3)
3–4	275 (26.1)	245 (24.2)	102 (23.0)	82 (26.3)
5 or more	385 (36.6)	330 (32.6)	130 (29.4)	107 (34.3)
Missing	5	2	1	1
Frequency of visits to doctor during past 4.5 years				
Rarely (<2/year)	146 (13.8)	53 (5.2)	22 (4.9)	15 (4.8)
2–8/year	576 (54.5)	504 (49.8)	220 (49.6)	162 (51.8)
>8/year	335 (31.7)	456 (45.0)	202 (45.5)	136 (43.5)
History of thyroid disease				
No	851 (80.5)	683 (67.4)	321 (72.3)	200 (63.9)
Yes	206 (19.5)	330 (32.6)	123 (27.7)	113 (36.1)
IRSD				
Most disadvantaged	356 (33.7)	353 (35.0)	160 (36.0)	101 (32.4)
Moderately disadvantaged	351 (33.2)	319 (31.6)	134 (30.2)	100 (32.1)
Least disadvantaged	349 (33.1)	338 (33.5)	150 (33.8)	111 (35.6)
Missing	1	3		1

^aOnly PTC cases with definitive *BRAF* results (*n* = 757) are reported in the table. Tumors that have papillary as first cancer and follicular as second cancer (*n* = 7) are not reported.

^bDate of diagnosis and date of consent are the reference dates for cases and controls, respectively.

BMI, body mass index; IQR, inter-quartile range; IRSD, index of relative socioeconomic disadvantage; PTC, papillary thyroid cancer.

TABLE 2. ASSOCIATION BETWEEN BODY MASS INDEX ONE YEAR BEFORE REFERENCE DATE AND RISK OF THYROID CANCER OVERALL AND BY SEX

Characteristics	Controls	Cases	Crude OR [CI]	Adjusted OR [CI]
BMI one year before reference date ^a				
Overall ^b	<i>n</i> = 1046 <i>n</i> (%)	<i>n</i> = 1003 <i>n</i> (%)		
Underweight/normal	438 (41.9)	312 (31.1)	1.00	1.00
Overweight	341 (32.6)	352 (35.1)	1.45 [1.18–1.78]	1.52 [1.22–1.90]
Obese	267 (25.5)	339 (33.8)	1.78 [1.44–2.21]	1.72 [1.37–2.16]
Per 5 kg/m ² increase in BMI				1.12 [1.04–1.20]
Female ^c	<i>n</i> = 777 <i>n</i> (%)	<i>n</i> = 723 <i>n</i> (%)		
Underweight/normal	360 (46.3)	259 (35.8)	1.00	1.00
Overweight	222 (28.6)	218 (30.2)	1.37 [1.07–1.75]	1.45 [1.12–1.87]
Obese	195 (25.1)	246 (34.0)	1.75 [1.37–2.24]	1.66 [1.28–2.16]
Per 5 kg/m ² increase in BMI				1.11 [1.03–1.20]
Male ^c	<i>n</i> = 269 <i>n</i> (%)	<i>n</i> = 280 <i>n</i> (%)		
Underweight/normal	78 (29.0)	53 (18.9)	1.00	1.00
Overweight	119 (44.2)	134 (47.9)	1.66 [1.08–2.54]	1.84 [1.17–2.89]
Obese	72 (26.8)	93 (33.2)	1.90 [1.19–3.03]	2.02 [1.23–3.31]
Per 5 kg/m ² increase in BMI				1.18 [0.99–1.41]

^aIn calculating ORs, 21 people were not included because of missing exposure or covariate information (complete-case analysis).

ORs were adjusted for:

^bAge, (continuous), sex, educational attainment (school only, technical training, university degree), smoking status (never, current, former), alcohol drinking status (never, current, former), number of walking sessions per week (0, 1–2, 3–4, 5 or more), history of benign thyroid disease (yes, no), and IRSD score (continuous).

^cAge (continuous), educational attainment (school only, technical training, university degree), smoking status (never, current, former), alcohol drinking status (never, current, former), number of walking sessions per week (0, 1–2, 3–4, 5 or more), history of benign thyroid disease (yes, no), and IRSD score (continuous).

CI, 95% confidence interval.

Discussion

Using a population-based sample of cases and controls, we found that being overweight or obese was associated with an increased risk of DTC risk in both women and men. Overall, the association with BMI did not vary with histological

features, but among those with PTC, the association with obesity was significantly stronger for *BRAF*-positive tumors than *BRAF*-negative tumors.

In accordance with Australian national cancer statistics (29), the median age of the case participants in our study was 52 years of age and there were almost three times as many

FIG. 1. Association between body mass index one year before reference date and risk of thyroid cancer according to (i) histological subtypes (papillary thyroid cancer and follicular thyroid cancer); (ii) *BRAF* mutational status; and (iii) presence or absence of potentially adverse histologic features in tumors (lymphovascular invasion, lymph node metastasis, or extra-thyroidal extension). CI, 95% confidence interval.

TABLE 3. ASSOCIATION BETWEEN BODY MASS INDEX ONE YEAR BEFORE REFERENCE DATE AND RISK OF PAPILLARY THYROID CANCER ACCORDING TO *BRAF* MUTATIONAL STATUS

Characteristics	Controls	BRAF-positive cases	BRAF-negative cases
BMI one year before reference date ^a			
Overall ^b	<i>n</i> = 1046 <i>n</i> (%)	<i>n</i> = 440 <i>n</i> (%)	Adjusted OR [CI] <i>n</i> = 309 <i>n</i> (%)
Underweight/normal	438 (41.9)	122 (27.7)	1.00
Overweight	341 (32.6)	152 (34.6)	1.74 [1.30–2.33]
Obese	267 (25.5)	166 (37.7)	2.20 [1.64–2.94]
Per 5 kg/m ² increase in BMI			1.18 [1.09–1.29]
Female ^c	<i>n</i> = 777 <i>n</i> (%)	<i>n</i> = 326 <i>n</i> (%)	<i>n</i> = 233 <i>n</i> (%)
Underweight/normal	360 (46.3)	102 (31.3)	1.00
Overweight	222 (28.6)	98 (30.1)	1.65 [1.18–2.30]
Obese	195 (25.1)	126 (38.7)	2.15 [1.54–2.98]
Per 5 kg/m ² increase in BMI			1.17 [1.07–1.29]
Male ^c	<i>n</i> = 269 <i>n</i> (%)	<i>n</i> = 114 <i>n</i> (%)	<i>n</i> = 76 <i>n</i> (%)
Underweight/normal	78 (29.0)	20 (17.5)	1.00
Overweight	119 (44.2)	54 (47.4)	2.08 [1.13–3.84]
Obese	72 (26.8)	40 (35.1)	2.38 [1.23–4.58]
Per 5 kg/m ² increase in BMI			1.24 [1.00–1.54]

^aControls (*n* = 1046) and only PTC cases with definitive *BRAF* results (*BRAF* positive—440; *BRAF* negative—309) are used in calculating ORs (complete-case analysis).

ORs were adjusted for:

^bAge (continuous), sex, educational attainment (school only, technical training, university degree), smoking status (never, current, former), alcohol drinking status (never, current, former), number of walking sessions per week (0, 1–2, 3–4, 5 or more), history of benign thyroid disease (yes, no), and IRSD score (continuous).

^cAge (continuous), educational attainment (school only, technical training, university degree), smoking status (never, current, former), alcohol drinking status (never, current, former), number of walking sessions per week (0, 1–2, 3–4, 5 or more), history of benign thyroid disease (yes, no), and IRSD score (continuous).

women as men. The prevalence of *BRAF* mutations in those with PTC in our study was 59%, which was similar to two small Australian single-center studies [57.9% of 95 cases (30) and 65% of 77 cases (31), respectively], but somewhat higher than that of a third study [44.7% of 76 cases (30)]. Globally, the estimated prevalence of the *BRAF* mutation in PTC has been somewhat lower than ours in the Czech Republic (32%) and United States (45.7%), but it was higher in Korea (78.5%) (18,30).

Our finding that obesity was associated with an increased risk of thyroid cancer overall is consistent with the results of most other studies (4,7,8,32–34). The observed increased risk associated with obesity (OR_{adj} = 1.72) was comparable to that reported in a pooled analysis of five U.S. cohort studies (thyroid cancer cases = 1156, hazard ratio [HR] = 1.53 [CI 1.31–1.79]) (7) but was higher than that reported in a larger pooled analysis (22 studies, thyroid cancer cases = 2825, HR = 1.09 [CI 0.98–1.22]), although that study included medullary thyroid cancers as well as DTC (33). Consistent with these studies, our sex-specific estimates also suggested a slightly weaker association with obesity in women than in men (7,33). Further, the 12% increase in risk of thyroid cancer per 5 kg/m² increase in BMI that we observed is consistent with the analyses of pooled prospective data (HRs = 1.17 and 1.06 per 5 kg/m² increase in BMI in two pooled studies, respectively) (7,33) and a large (5.2 million people) UK population-based cohort (HR = 1.09 per 5 kg/m² increase in BMI) (32).

Consistent with other studies (33,35), we found no clear difference in the association with obesity according to

histological subtypes (PTC vs. FTC), although the relatively small number of FTC cases (*n* = 89) in our study limits definitive conclusions. We observed that people with a higher BMI had greater likelihood of *BRAF*-mutated PTC, which is more likely to be associated with poorer prognostic features such as extrathyroidal tumor extension and lymph node metastasis (15,16,36,37). Two previous hospital-based case-only studies from Korea (*n* = 1121) (18) and China (*n* = 104) (19) have also reported positive associations between higher BMI and *BRAF*-mutated PTC, although methodological differences preclude direct comparison of estimates. In addition, a temporal analysis of molecular characteristics of PTC tumors (*n* = 228) reported an increase in *BRAF* mutation prevalence over time (43% in 1996–2000 and 88% in 2001–05), suggesting that this rise might contribute to an increase in thyroid cancer incidence (38). However, participants' BMI was not analyzed. Why the BMI–thyroid cancer association might differ by *BRAF* mutational subtype is uncertain. If other cancers with frequent *BRAF* mutations such as colorectal cancer, melanoma, and ovarian serous borderline cancers had similar associations, it could imply a common underlying mechanism. However, published data are only available for colorectal cancer and these did not show that the obesity–colorectal cancer association varied by *BRAF* mutational status (17,39). Thus, the mechanism may depend on the primary site of the cancer and levels of related hormones. Obesity increases thyrotropin levels, which, in turn, influence follicular cell growth, differentiation, and proliferation, and thus potentially promote gene alterations such as the *BRAF* mutation (19,40).

Based on the association between *BRAF* mutation and adverse thyroid cancer characteristics, we had expected that the association between obesity and DTC might differ according to the presence or absence of adverse features in tumors, but this was not the case. This may be because while most cancers with adverse features had *BRAF* mutations, these mutations were also moderately common in cancers without these features. Since the *BRAF* mutation is an early event in thyroid carcinogenesis (14,41), localized tumors that were *BRAF* positive may have been diagnosed before the tumors developed these features.

Our results indicate that frequent consultations with medical practitioners among those who were overweight or obese explained some of the association between overweight/obesity and thyroid cancer diagnosis, perhaps due to the greater opportunity for incidental diagnosis of indolent thyroid cancers. We do not have information on the reasons that people consulted doctors, but most of the cases' excess visits occurred in the six months before diagnosis, and some may have been related to the diagnostic workup for their thyroid cancer. When services accessed in the six months before the reference date were excluded, the proportion mediated was reduced. Nonetheless, a larger proportion of the association between overweight/obesity and *BRAF*-negative PTC was mediated by frequent consultations with medical practitioners compared with the *BRAF*-positive PTC. These *BRAF*-stratified results suggest that the increased risk of thyroid cancer associated with obesity is unlikely to be solely due to over-diagnosis.

One limitation of our study is the potential for selection bias due to the relatively low participation proportion among those approached to take part in our study as controls. We tested the possible effect of this by using a QBA, which suggested that we may have overestimated the overall obesity–thyroid cancer association. However, we calculated BMI by using self-reported weight and height, which generally underestimates true BMI (11,42), whereas BMI for the general Queensland population was calculated based on measured height and weight. This might partially explain the difference in BMI distribution between our controls and the general Queensland population and, thus, we could be overestimating the effect of selection bias. In addition, ~30% of potentially eligible cases did not participate and further analyses, conservatively assuming that the proportion of nonparticipating cases who were obese was 5% higher than among participating cases, also suggested that the degree of bias is likely to be lower than we estimated. Further, our main findings are very similar to those from large cohort studies, suggesting that selection bias is unlikely to account entirely for the observed association, particularly among women. Another limitation may be that we used immunohistochemistry staining to determine *BRAF* mutation status of the PTC tumors rather than DNA sequencing. However, the sensitivity and specificity of immunohistochemistry staining for *BRAF* mutation are high (43), so misclassification is likely minimal. Finally, recall bias could also have affected our results although it is unlikely that the participants were aware of the potential association between body size and thyroid cancer at the time of interview.

In conclusion, our findings suggest that obesity is associated with an increased risk of DTC, particularly with *BRAF*-mutated PTC in women. It is unlikely that the obesity-thyroid

cancer association previously reported in the literature is purely an artifact of over-ascertainment due to greater use of medical services by those who are obese. The increasing proportion of overweight and obesity in many populations across the world may, therefore, be contributing, at least in small part, to rising thyroid cancer incidence.

Acknowledgments

The authors would like to acknowledge the work of Andrea McMurtrie, RN, and Emily Dickson, BA, for undertaking the data collection; the Royal Brisbane and Women's Hospital's assistance with participant recruitment; and Sullivan Nicolaides Pathology for their assistance with the *BRAF* mutation data.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This study was supported by a grant from the National Health and Medical Research Council (NHMRC APP1047733) of Australia. S.J.J. and R.E.N. were supported by fellowships from the NHMRC (APPs 1061341 and 1060183 respectively). S.T.R. is supported by a scholarship from the QIMR Berghofer Medical Research Institute.

Supplementary Material

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